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Effects of dietary selenium on host response to necrotic enteritis in young broilers

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ABSTRACT

The effects of dietary supplementation of young broiler chickens with an organic selenium (Se) formulation, B-Traxim Se, on experimental necrotic enteritis (NE) were studied. Chickens treated with three Se doses (0.25, 0.50, 1.00 mg/kg) from hatch were orally challenged with Eimeria maxima at 14 days of age followed by Clostridium perfringens to induce NE. Chickens fed with 0.50 mg/kg Se showed significantly increased body weights and antibody levels against NetB, and significantly reduced gut lesions compared with non-supplemented chickens. However, there were no significant differences in Eimeria oocyst shedding between the Se-treated and non-supplemented groups. Levels of IL-1β, IL-6, IL-8, iNOS, LITAF, TNFSF15, AvBD6, AvBD8, and AvBD13 transcripts were increased in the gut and spleen of at least one of the three Se-treated groups compared with the non-treated group. These results suggest that dietary supplementation of young broilers with Se might be beneficial to reduce the negative consequence of NE.

1. Introduction

Necrotic enteritis (NE) is a devastating enteric disease of poultry that results in more than $2 billion economic losses through growth depression, and high morbidity and mortality (Park et al., 2008; Timbermont et al., 2011). NE is caused by the anaerobic bacterium *Clostridium perfringens*, but it is difficult to experimentally reproduce the disease with *C. perfringens* alone because of various risk factors involved in the development of NE. Co-infection with *Eimeria maxima* has been commonly associated with NE (Lee et al., 2011b). With the emergence of antibiotic-resistant pathogens and increasing concerns about chemical residues in poultry meat, many antibiotics which have been traditionally used to prevent and control NE have been restricted or banned. Therefore, there is an urgent need to develop antibiotic alternative methods for reducing economic losses due to NE. Several alternative approaches to antibiotics have been proposed to reduce the negative consequences of NE in broilers, including prebiotics, probiotics, functional feed additives (yeast products, organic acids, essential oils, and phytonutrients), and vaccines against *C. perfringens* toxins (Fernando et al., 2011; Geier et al., 2010; Jerzsele et al., 2012; Lee et al., 2013; Lillehoj and Lee, 2012; Mot et al., 2013).

Selenium (Se) is an essential trace element that plays important roles in immune function, health, and animal productivity (Yoon et al., 2007). Sodium selenite and sodium selenate are the most common inorganic Se sources used in livestock feeds (Yuan et al., 2012). In prior studies, inorganic Se showed an anti-cryptosporidial effect (Huang and Yang, 2002) and promoted protective immunity against *Eimeria tenella* (Colnago et al., 1984). Recently, however, there has been interest in organic Se sources, such as Se-enriched yeast and selenomethionine. Organic Se generally shows higher efficacy and bioavailability and less toxicity compared with inorganic Se (Briens et al., 2013). A new organic Se formulation, B-Traxim Se (Pancosma SA, Geneva, Switzerland), is formed by the incorporation of inorganic Se into soybean protein, which is subsequently hydrolyzed to form a commercial product specifically designed for animal feeding (Pavlata et al., 2012). A previous study reported that breeder hens fed B-Traxim Se showed high levels of Se in eggs and lower levels of glutathione peroxidase in liver than those fed sodium selenite (Leeson et al., 2008). The objective of the present study was to evaluate the effects of different levels of dietary B-Traxim Se in broilers on host protective response against experimental NE using an *E. maxima*/*C. perfringens* co-infection model system (Park et al., 2008).

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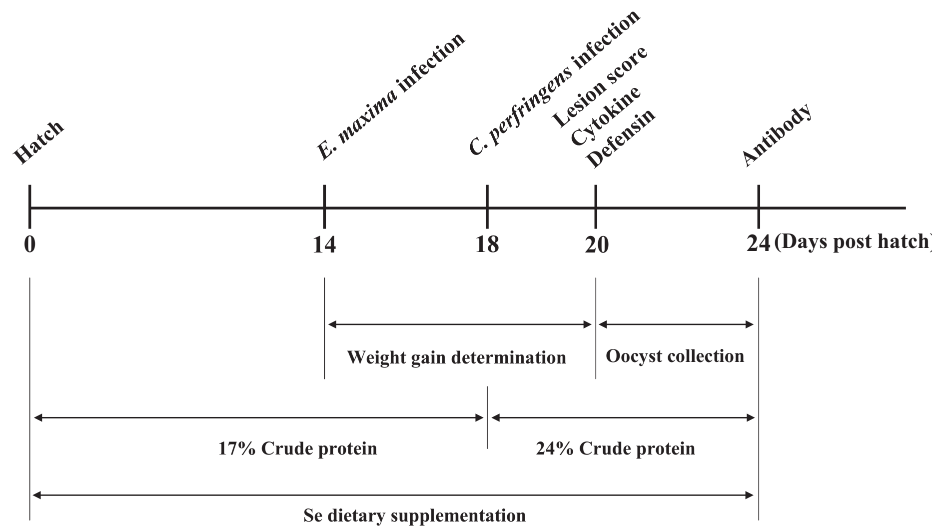

Fig. 1. Schematic outline of the experimental design.

2. Materials and methods

2.1. Experimental design

A schematic diagram of the experimental protocol is shown in Fig. 1. One-day-old Ross broiler chickens (Longenecker's Hatchery, Elizabethtown, PA) were housed in Petersime starter brooder units and provided with an antibiotic-free starter diet (Table 1) between days 1 and 18 and a grower diet (Table 1) after day 18 until the end of trial. Both basal diets include 0.10 mg Se/kg diet. Feed and water were given *ad libitum*. Chickens were randomly divided into five groups (15 chickens/group): uninfected control, infected and non-supplemented control, and infected and supplemented groups with 0.25, 0.50, or 1.00 mg/kg of B-Traxim Se. All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Beltsville Agricultural Research Center.

2.2. Experimental NE model

Chickens were reared in brooder pens in an *Eimeria*-free facility for 14 days post-hatch and then transferred into large hanging cages (3 birds/cage) at a separate location where they were infected and kept until the end of the experiment. At 14 days of age, chickens were orally infected with *E. maxima* Beltsville strain 41A (1.0×10^4 sporulated oocysts/bird), followed by *C. perfringens* strain Del-1 (1.0×10^9 CFU/bird) at 18 days of age. To facilitate the development of NE, the diet formulation was changed from the starter diet to the grower diet after day 18.

Table 1
Ingredient and nutrition of the basal diets.[a]

Ingredients[b] (g/kg)	Starter diet (days 0–18)	Grower diet (days 18–24)
Crude protein	170.0	240.0
Carbohydrate	610.0	540.0
Selenium-free mineral and vitamin mixture	150.0	150.0
Fat	47.0	47.0
Fiber	24.0	24.0
Selenium[c] (mg/kg)	0.10	0.10

[a] Based on the producer's declaration that B-Traxim Se contains 1.1% (wt/wt) Se metal content, the basal diets were added to the B-Traxim Se (Pancosma S.A., Geneva) to prepare the 0.25, 0.50, and 1.00 mg/kg Se supplemented diets by calculation.
[b] Data were from USDA/FeedMill, Beltsville, MD.
[c] The Se concentration was calculated.

2.3. Evaluation of body weight gain, lesion score, and oocyst shedding

Body weight gains were measured between 0 and 6 days post-infection with *E. maxima*. Intestinal lesion scores were evaluated at 6 days post-infection with *E. maxima* on a scale of 0 (none) to 4 (high) in a blinded fashion by three independent observers as described earlier (Prescott, 1979). Fecal oocysts were individually enumerated between 6 and 10 days post-infection with *E. maxima* using a McMaster chamber as described (Jang et al., 2010).

2.4. Determination of serum antibody levels against C. perfringens *toxins*

Blood samples (4 birds/group) were collected by cardiac puncture at 6 days post-infection with *C. perfringens* following euthanasia. Sera were prepared by low speed centrifugation and serum antibodies against α-toxin and NetB toxin were measured by enzyme-linked immunosorbent assay (ELISA) as described (Lee et al., 2011c) using recombinant *C. perfringens* α-toxin and NetB toxin expressed in *Escherichia coli* as described (Lee et al., 2011a). Briefly, 96-well microtiter plates were coated overnight with 1.0 μg/well of purified recombinant toxin proteins, washed with PBS containing 0.05% Tween 20 (PBS-T), and blocked with PBS containing 1% BSA for 1 h at room temperature. Diluted serum samples (1:20, 100 μl/well) were added and incubated with gentle shaking for 2 h at room temperature. The plates were washed with PBS-T and bound antibodies were detected with peroxidase-conjugated rabbit anti-chicken IgG and peroxidase-substrate (Sigma, St. Louis, MO). The optical density (OD) at 450 nm was determined with an automated microtiter reader (Bio-Rad, Richmond, CA). All samples were analyzed in quadruplicate.

2.5. Quantification of pro-inflammatory cytokines and AvBD transcript levels

The levels of transcripts for pro-inflammatory cytokines and AvBD were measured as described (Hong et al., 2006; Kim et al., 2008) by quantitative RT-PCR. At 2 days post-infection with *C. perfringens*, the spleen and jejunum located proximal to the Meckel's diverticulum

Table 2
Oligonucleotide primers used for quantitative RT-PCR.

RNA target	Primer sequence	PCR product size (bp)	Accession No.
GAPDH	F: 5′-GGTGGTGCTAAGCGTGTTAT-3′ R: 5′-ACCTCTGTCATCTCTCCACA-3′	264	NM_204305.1
IL-1β	F: 5′-TGGGCATCAAGGGCTACA-3′ R: 5′-TCGGGTTGGTTGGTGATG-3′	244	NM_204524.1
IL-6	F: 5′-CAAGGTGACGGAGGAGGAC-3′ R: 5′-TGGCGAGGAGGGATTTCT-3′	254	NM_204628.1
IL-8	F: 5′-GGCTTGCTAGGGGAAATGA-3′ R: 5′-AGCTGACTCTGACTAGGAAACTGT-3′	200	NM_205498.1
iNOS	F: 5′-TGGGTGGAAGCCGAAATA-3′ R: 5′-GTACCAGCCGTTGAAAGGAC-3′	241	U46504.1
LITAF	F: 5′-TGTGTATGTGCAGCAACCCGTAGT-3′ R: 5′-GGCATTGCAATTTGGACAGAAGT-3′	229	AY765397.1
TNFSF15	F: 5′-CCTGAGTATTCCAGCAACGCA-3′ R: 5′-ATCCACCAGCTTGATGTCACTAAC-3′	292	NM_001024578.1
AvBD6	F: 5′-ATCCTTTACCTGCTGCTGTCTGT-3′ R: 5′-GAGGCCATTTGGTAGTTGC-3′	250	NM_001001193.1
AvBD8	F: 5′-TGTGGCTGTTGTGTTTTGT-3′ R: 5′-CTGCTTAGCTGGTCTGAGG-3′	267	NM_001001781.1
AvBD13	F: 5′-CATCGTTGTCATTCTCCTCCTC-3′ R: 5′-GGTGGAGAACCTGCAGCAGCG-3′	163	NM_001001780.1

F = forward primer, R = reverse primer.

were collected from 4 chickens/group. The jejunums were cut longitudinally and washed three times with ice-cold Hanks' balanced salt solution (HBSS) containing 100 U/ml of penicillin and 100 μg/ml of streptomycin (Sigma). The mucosal layer was carefully scraped away using a surgical scalpel (Nunc, Thermo Fisher Scientific Inc., Roskilde, Denmark) and intraepithelial lymphocytes (IELs) were isolated by density gradient centrifugation. Total RNA from intestine and spleen was extracted using TRIzol (Invitrogen, Carlsbad, CA). Five micrograms of total RNA were treated with 1.0 U of DNase I in 1.0 μl of 10 × reaction buffer (Sigma) for 15 min at room temperature, 1.0 μl of stop solution was added, and the mixture was heated at 70 °C for 10 min. RNA was reverse-transcribed using the StrataScript first-strand synthesis system (Stratagene, La Jolla, CA) according to the manufacturer's recommendations. Quantitative RT-PCR oligonucleotide primers for IL-1β, IL-6, IL-8, TNFSF15, LITAF, iNOS, AvBD6, AvBD 8, AvBD13, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control are listed in Table 2. Amplification and detection were carried out using equivalent amounts of total RNA with the Mx3000P system and Brilliant SYBR Green QPCR master mix (Stratagene). Standard curves were generated using $\log_{10}$ diluted standard RNA and the levels of individual transcripts were normalized to those of GAPDH by the Q-gene program (Muller et al., 2002). To normalize RNA levels between samples within an experiment, the mean threshold cycle (C_t) values for the amplification products were calculated by pooling values from all samples in that experiment. Each sample was analyzed in triplicate.

2.6. Statistical analysis

All data expressed as the mean ± SD values were subjected to one-way ANOVA using SPSS software (SPSS 15.0 for Windows, Chicago, IL), and compared by the Duncan's multiple range tests. Differences between means were considered statistically significant at $P < 0.05$.

3. Results

3.1. Effect of dietary Se on body weight gain, lesion score, and oocyst shedding

Chickens fed with 0.50 and 1.00 mg/kg Se showed significantly ($P < 0.05$) increased body weight gain between 0 and 6 days post-infection with *E. maxima* compared with the infected and non-supplemented control group (Fig. 2A). As shown in Fig. 2B, intestinal lesion scores were significantly reduced ($P < 0.05$) in the 0.25 and 0.50 mg/kg Se groups at 6 days post-infection with *E. maxima* compared with the infected and non-supplemented control group. No differences in fecal oocyst shedding were seen in any of the experimental groups (Fig. 2C). As expected, the infected control group showed depression, diarrhea, and reduced body weigh gain compared with the uninfected control because of severe intestinal lesion resulting from coinfection with *E. maxima* and *C. perfringens*.

3.2. Effect of dietary Se on toxin specific serum antibody levels

At 6 days post-infection with *C. perfringens*, no differences in serum antibody levels against α-toxin were seen in any of the experimental groups (Fig. 3A). However, serum antibody levels against Net-B toxin were significantly increased ($P < 0.05$) in the 0.25 and 0.50 mg/kg Se groups compared with the infected and non-supplemented control group (Fig. 3B).

3.3. Effect of dietary Se on pro-inflammatory cytokine transcript levels

The levels of transcripts for IL-1β, IL-6, IL-8, LITAF, TNFSF15, and iNOS in the intestine and spleen were significantly increased ($P < 0.05$) in at least one of the three infected and Se-supplemented groups compared with the infected and non-supplemented control group (Fig. 4). In the case of intestinal IL-1β, IL-6, and IL-8 and splenic IL-1β, IL-8, and LITAF, transcript levels were increased in all three Se-supplemented groups compared with the non-supplemented controls group, although a consistent dose–response effect was not apparent for any of these transcripts.

3.4. Effect of dietary Se on AvBD transcript levels

The levels of transcripts for AvBD6, AvBD8, and AvBD13 in the intestine and spleen were significantly increased ($P < 0.05$) in at least one of the three infected and Se-supplemented groups compared with the infected and non-supplemented

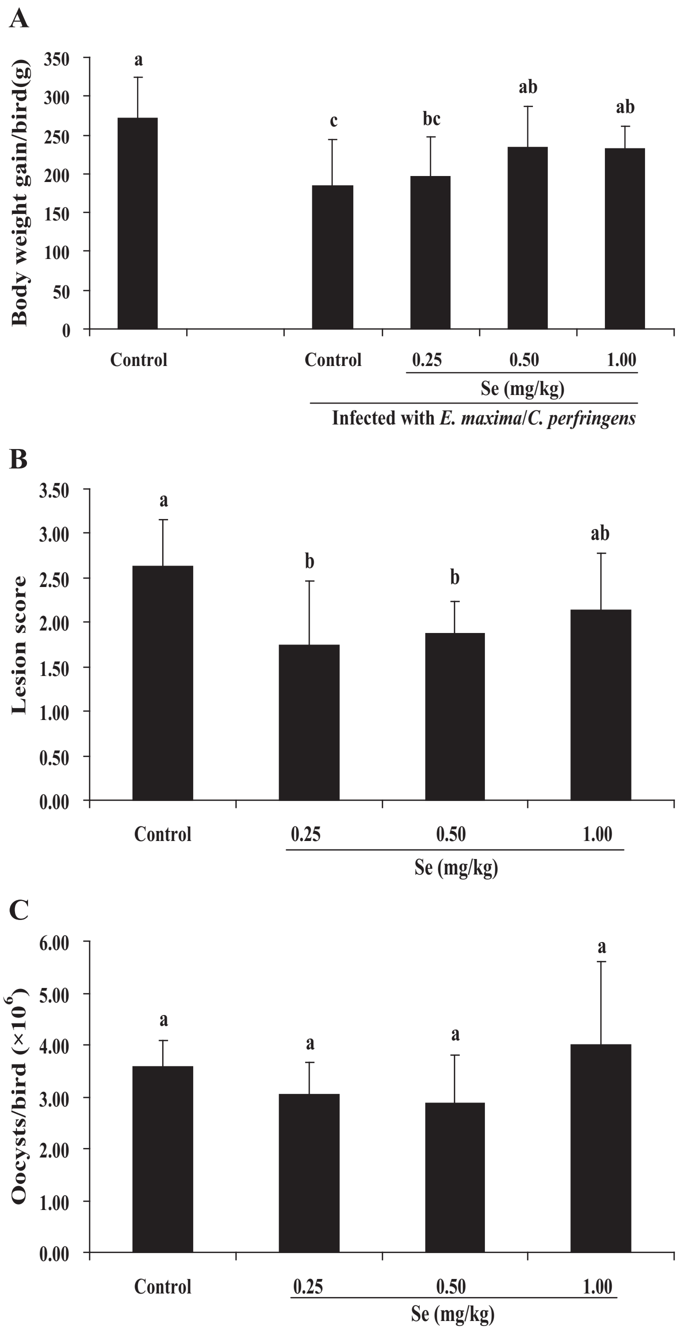

Fig. 2. Effects of dietary Se on body weight gain, intestinal lesion scores, and fecal oocyst shedding. Chickens were fed with a non-supplemented diet or diets supplemented with 0.25, 0.50, or 1.00 mg/kg B-Traxim™ Se from hatch, and uninfected or orally co-infected with 1.0×10^4 sporulated oocysts of *E. maxima* at 14 days of age followed by *C. perfringens* infection with 1.0×10^9 CFU at 18 days of age. (A) Body weight gains were calculated between 0 and 6 days post-infection with *E. maxima*. (B) Intestinal lesion scores were assessed at 6 days post-infection with *E. maxima* on a scale of 0 (none) to 4 (high). (C) Fecal oocyst numbers were measured between 6 and 10 days post-infection with *E. maxima*. Each bar represents the mean ± SD value ($n = 12$). Bars with different letters are significantly different according to the Duncan's multiple range test ($P < 0.05$).

control group (Fig. 5). In the case of splenic AvBD8, transcript levels were increased in all three Se-supplemented groups compared with the non-supplemented controls group, and in the case of intestinal AVBD13 transcripts, a dose–response effect was evident.

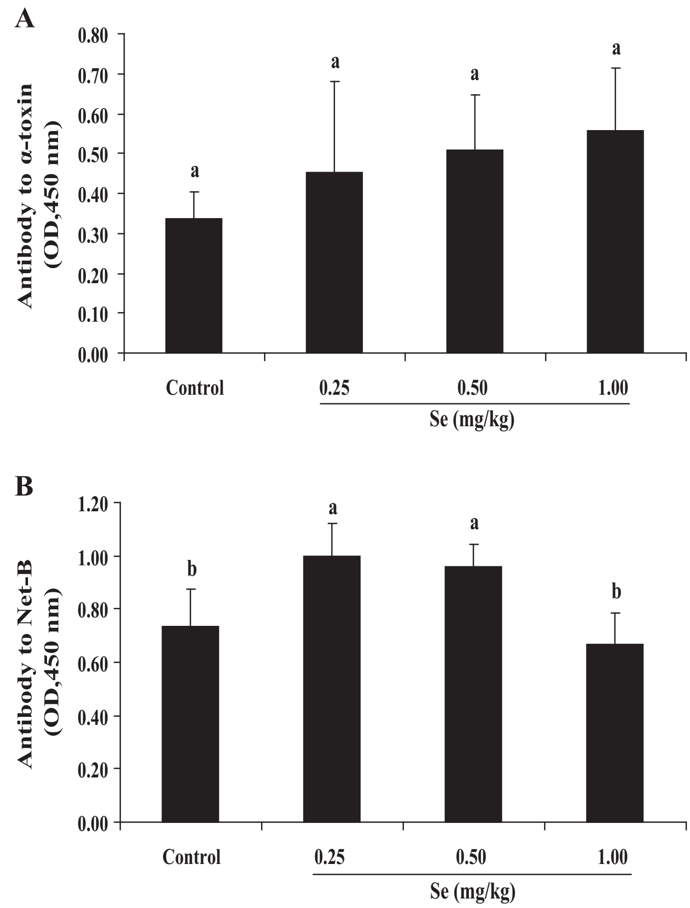

Fig. 3. Effects of dietary Se on serum antibody levels against *C. perfringens* α-toxin and Net-B toxin. Chickens were fed with a non-supplemented diet or diets supplemented with 0.25, 0.50, or 1.00 mg/kg B-Traxim™ Se from hatch, and uninfected or orally co-infected with 1.0×10^4 sporulated oocysts of *E. maxima* at 14 days of age followed by *C. perfringens* infection with 1.0×10^9 CFU at 18 days of age. Serum antibody levels against α-toxin (A) and Net-B toxin (B) were measured by ELISA at 6 days post-infection with *C. perfringens*. Each bar represents the mean ± SD value ($n = 4$). Bars with different letters are significantly different according to the Duncan's multiple range tests ($P < 0.05$).

4. Discussion

Se is an essential micronutrient that plays important roles in immune function in humans and animals. Previous researches showed that Se deficiency impaired the cellular and humoral immune function and Se supplementation induced favorable effects on T cell responses and antibody synthesis in chickens (Marsh et al., 1981; Peng et al., 2011a, 2011b, 2012). In the present study, broiler chickens fed with appropriate B-Traxim Se showed increased body weight gain, reduced intestinal lesions, improved serum antibody to Net-B toxin, and up-regulated transcript levels of IL-1β, IL-6, IL-8, LITAF, TNFSF15, iNOS, AvBD6, AvBD8 and AvBD13 compared with the unsupplemented and infected chickens using an experimental model of NE, which was consistent with our previous study evaluating the effects of *in ovo* injection with Se on immune and antioxidant responses during experimental NE in commercial broilers (Lee et al., 2014). The immune protection from Se supplementation might be attributable to improved intestinal physiology allowing for greater nutrient absorption and enhanced immune response against NE infection.

It is well known that Se may be toxic when supplemented in excess of the biologically required levels. To ensure feed safety, maximum Se levels in complete poultry feed have been set at 0.5 mg/kg in the European Union (2004) and China (Ministry of

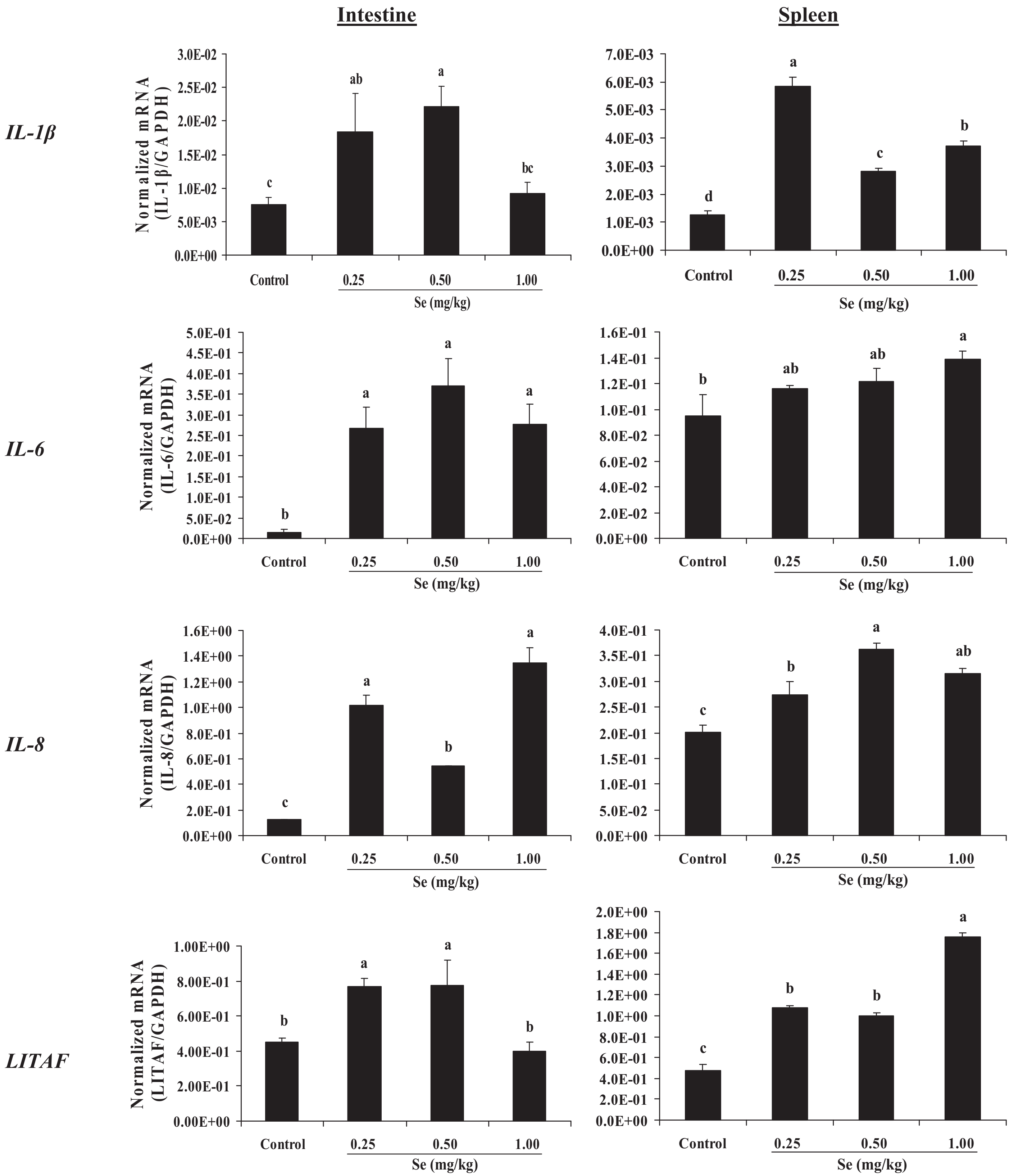

Fig. 4. Effects of dietary Se on the levels of transcripts for pro-inflammatory cytokines. Chickens were fed with a non-supplemented diet or diets supplemented with 0.25, 0.50, or 1.00 mg/kg B-Traxim™ Se from hatch, and uninfected or orally co-infected with 1.0×10^4 sporulated oocysts of *E. maxima* at 14 days of age followed by *C. perfringens* infection with 1.0×10^9 CFU at 18 days of age. Levels of transcripts for IL-1β, IL-6, IL-8, iNOS, LITAF, and TNFSF15 in the intestine and spleen were measured by quantitative RT-PCR at 2 days post-infection with *C. perfringens* and normalized to GAPDH transcript levels. Each bar represents the mean ± SEM value ($n = 4$). Bars with different letters are significantly different ($P < 0.05$) according to the Duncan's multiple range tests.

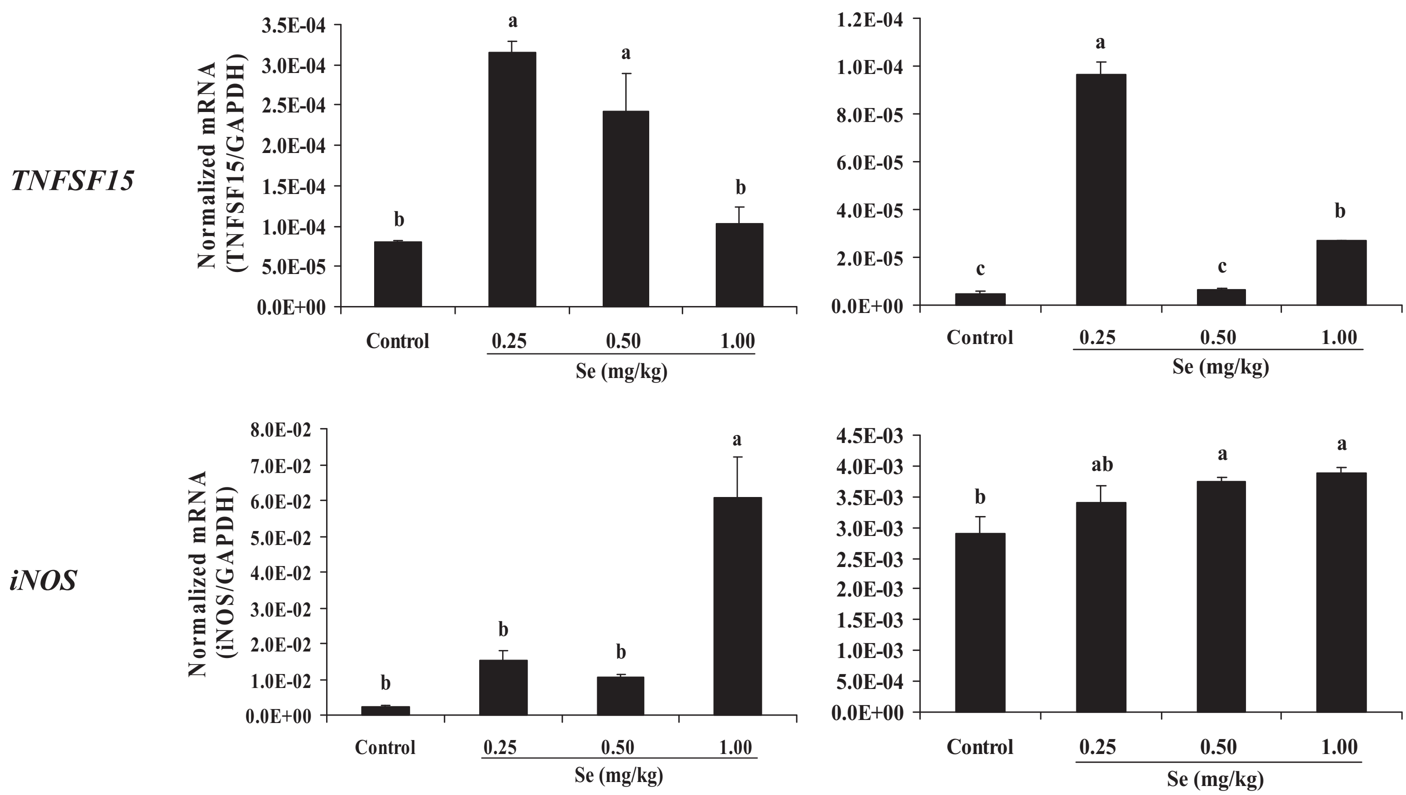

Fig. 4. (*continued*)

Agriculture, 2010), and 2.0 mg/kg in the United States (AAFCO, 2011) (Cai et al., 2012). In the current study the broiler chickens fed with 0.50 mg/kg B-Traxim Se showed the best protective effects. The protective effects of Se did not consistently increase proportionally with the increased dose of supplemented Se between 0.25 and 1.00 mg/kg possibly because the highest dose may be influenced by toxic effects in this particular broiler group.

C. perfringens α-toxin and NetB toxin are major virulence factors implicated in the pathogenesis of NE and are considered to be potential vaccine candidates in chickens (Fernandes da Costa et al., 2013; Jang et al., 2012; Sumners et al., 2012). The antibody levels against α-toxin and NetB toxin may be relevant in protective host immunity against *C. perfringens* (Lee et al., 2012). Our observations that serum antibody levels against these two toxins increased in chickens supplemented with 0.25 and 0.50 mg/kg Se indicate a beneficial effect of Se on humoral immunity in NE-inflicted broilers. A similar relationship between Se levels and antibody responses was reported in a previous study (Cai et al., 2012).

Pro-inflammatory cytokines and defensins represent important components of innate immunity during the early phase of the host response to pathogens (Ramasamy et al., 2012). The role of pro-inflammatory cytokines in the host response during experimental avian NE is well-established, although the role of AvBDs is less clear (Lee et al., 2011b). However, up-regulation of AvBD expression was associated with increased resistance of chickens to infection by *Salmonella enterica*, *E. coli*, and *C. perfringens* (Crhanova et al., 2011; Ferro et al., 2004), and human defensins show parasiticidal activity against *Trypanosoma cruzi*, *Cryptosporidium parvum*, and *Toxoplasma gondii* (Carryn et al., 2012; Madison et al., 2007; Tanaka et al., 2010). Our previous study showed that IL-1β, IL-6, IL-17F, TNFSF15, AvBD6, AvBD8, and AvBD13 were all highly induced in broilers co-infected model with *E. maxima* and *C. perfringens* (Hong et al., 2012), suggesting that these effector molecules play a critical role in host defense against NE. Based on the results of this study, we hypothesize that dietary B-Traxim Se supplementation stimulates the production of pro-inflammatory cytokines and AvBDs to exert direct and indirect coccidiocidal and bactericidal effects against *E. maxima* and *C. perfringens*. In this study the results that chickens supplemented with Se showed greater IL-1β, IL-6, IL-8, TNFSF15, LITAF, iNOS, AvBD6, AvBD 8, and AvBD13 transcript levels compared with the unsupplemented and infected chickens supported our hypothesis. However, there was no consistent dose–response relationship between increasing dietary Se concentration and the particular parameter measured, so it can be speculated that some additional factors such as the relative kinetics of expression and activities of the individual cytokines and AvBDs, the local availability of Se in target tissues and organs may influence the observed outcomes.

In summary, the present study revealed that dietary supplementation of young broilers with B-Traxim Se enhanced host protection against experimental avian NE that was associated with up-regulated expression of pro-inflammatory cytokines and AvBDs. Further studies are required to identify the molecular and cellular mechanisms through which this organic Se formulation enhances resistance against this disease.

Acknowledgments

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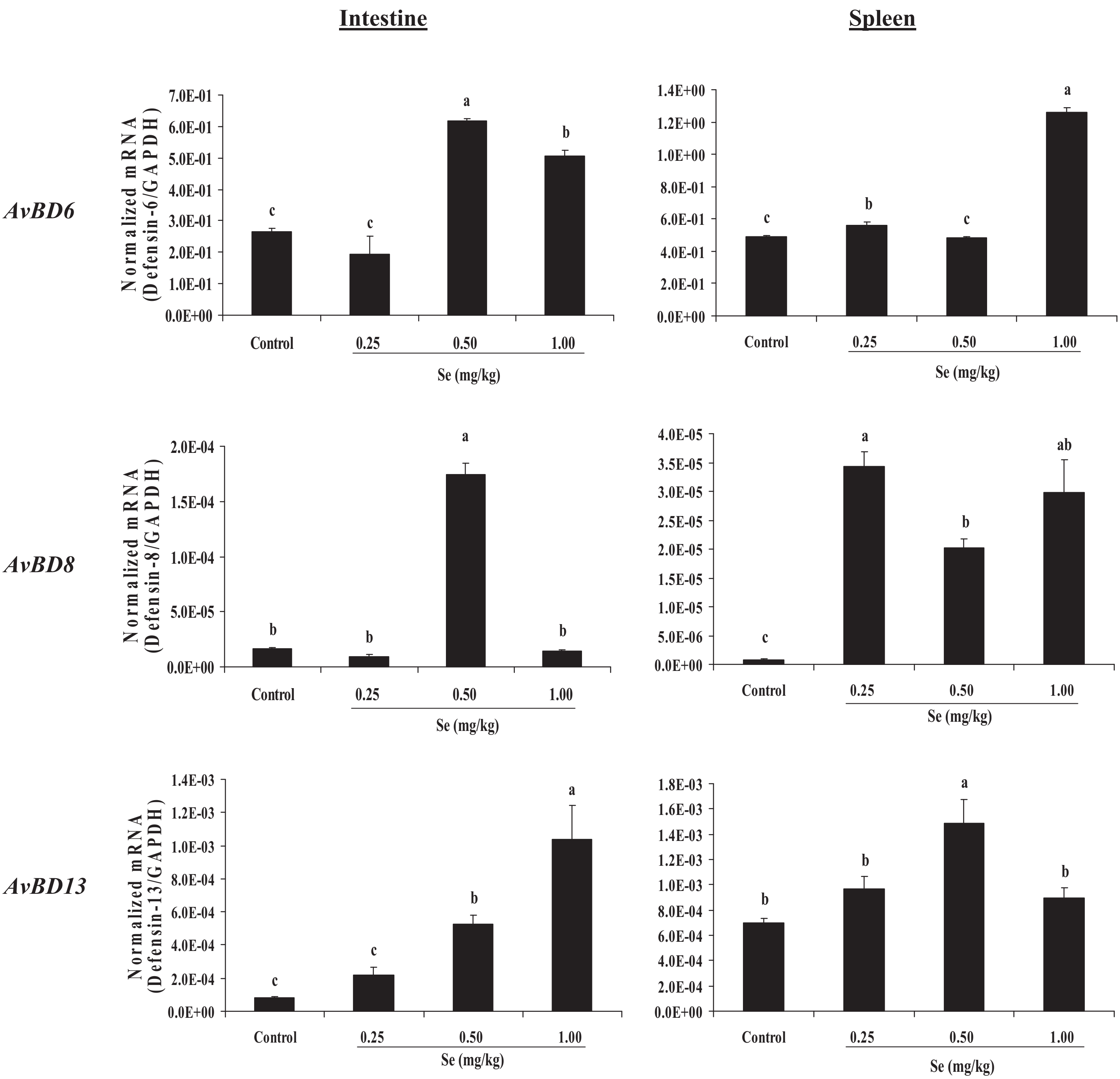

Fig. 5. Effects of dietary Se on the levels of transcripts for AvBDs. Chickens were fed with a non-supplemented diet or diets supplemented with 0.25, 0.50, or 1.00 mg/kg B-Traxim™ Se from hatch, and uninfected or orally co-infected with 1.0×10^4 sporulated oocysts of *E. maxima* at 14 days of age and *C. perfringens* infection with 1.0×10^9 CFU at 18 days of age. The levels of transcripts for AvBD6, AvBD8, and AvBD13 in the intestine and spleen were measured by quantitative RT-PCR at 2 days post-infection with *C. perfringens* and normalized to GAPDH transcript levels. Each bar represents the mean ± SEM value ($n = 4$). Bars with different letters are significantly different ($P < 0.05$) according to the Duncan's multiple range tests.

SeungIk Jang, Misun Jeong and SeungKyoo Lee, USDA-ARS, Beltsville, MD, for their valuable contributions to this study.

References

AAFCO, 2011. AAFCO Model Guidance Document. Official guidelines suggested for contaminants in individual mineral feed ingredients. Official publication. Association of American Feed Control Officials Inc., Olympia, WA.

Briens, M., Mercier, Y., Rouffineau, F., Vacchina, V., Geraert, P.A., 2013. Comparative study of a new organic selenium source v. seleno-yeast and mineral selenium sources on muscle selenium enrichment and selenium digestibility in broiler chickens. British Journal of Nutrition 110, 617–624.

Cai, S.J., Wu, C.X., Gong, L.M., Song, T., Wu, H., Zhang, L.Y., 2012. Effects of nano-selenium on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers. Poultry Science 91, 2532–2539.

Carryn, S., Schaefer, D.A., Imboden, M., Homan, E.J., Bremel, R.D., Riggs, M.W., 2012. Phospholipases and cationic peptides inhibit Cryptosporidium parvum sporozoite infectivity by parasiticidal and non-parasiticidal mechanisms. Journal of Parasitology 98, 199–204.

Colnago, G.L., Jensen, L.S., Long, P.L., 1984. Effect of selenium and vitamin E on the development of immunity to coccidiosis in chickens. Poultry Science 63, 1136–1143.

Crhanova, M., Hradecka, H., Faldynova, M., Matulova, M., Havlickova, H., Sisak, F., et al., 2011. Immune response of chicken gut to natural colonization by gut microflora and to Salmonella enterica serovar enteritidis infection. Infection and Immunity 79, 2755–2763.

European Union, 2004. List of the authorized additives in feedingstuffs published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs.
Fernandes da Costa, S.P., Mot, D., Bokori-Brown, M., Savva, C.G., Basak, A.K., Van Immerseel, F., et al., 2013. Protection against avian necrotic enteritis after immunisation with NetB genetic or formaldehyde toxoids. Vaccine 31, 4003–4008.
Fernando, P.S., Rose, S.P., Mackenzie, A.M., Silva, S.S., 2011. Effect of diets containing potato protein or soya bean meal on the incidence of spontaneously-occurring subclinical necrotic enteritis and the physiological response in broiler chickens. British Poultry Science 52, 106–114.
Ferro, P.J., Swaggerty, C.L., Kaiser, P., Pevzner, I.Y., Kogut, M.H., 2004. Heterophils isolated from chickens resistant to extra-intestinal Salmonella enteritidis infection express higher levels of pro-inflammatory cytokine mRNA following infection than heterophils from susceptible chickens. Epidemiology and Infection 132, 1029–1037.
Geier, M.S., Mikkelsen, L.L., Torok, V.A., Allison, G.E., Olnood, C.G., Boulianne, M., et al., 2010. Comparison of alternatives to in-feed antimicrobials for the prevention of clinical necrotic enteritis. Journal of Applied Microbiology 109, 1329–1338.
Hong, Y.H., Lillehoj, H.S., Lillehoj, E.P., Lee, S.H., 2006. Changes in immune-related gene expression and intestinal lymphocyte subpopulations following Eimeria maxima infection of chickens. Veterinary Immunology and Immunopathology 114, 259–272.
Hong, Y.H., Song, W., Lee, S.H., Lillehoj, H.S., 2012. Differential gene expression profiles of beta-defensins in the crop, intestine, and spleen using a necrotic enteritis model in 2 commercial broiler chicken lines. Poultry Science 91, 1081–1088.
Huang, K., Yang, S., 2002. Inhibitory effect of selenium on Cryptosporidium parvum infection in vitro and in vivo. Biological Trace Element Research 90, 261–272.
Jang, S.I., Lillehoj, H.S., Lee, S.H., Lee, K.W., Park, M.S., Bauchan, G.R., et al., 2010. Immunoenhancing effects of Montanide ISA oil-based adjuvants on recombinant coccidia antigen vaccination against Eimeria acervulina infection. Veterinary Parasitology 172, 221–228.
Jang, S.I., Lillehoj, H.S., Lee, S.H., Lee, K.W., Lillehoj, E.P., Hong, Y.H., et al., 2012. Vaccination with Clostridium perfringens recombinant proteins in combination with Montanide ISA 71 VG adjuvant increases protection against experimental necrotic enteritis in commercial broiler chickens. Vaccine 30, 5401–5406.
Jerzsele, A., Szeker, K., Csizinszky, R., Gere, E., Jakab, C., Mallo, J.J., et al., 2012. Efficacy of protected sodium butyrate, a protected blend of essential oils, their combination, and Bacillus amyloliquefaciens spore suspension against artificially induced necrotic enteritis in broilers. Poultry Science 91, 837–843.
Kim, D.K., Lillehoj, H.S., Hong, Y.H., Park, D.W., Lamont, S.J., Han, J.Y., et al., 2008. Immune-related gene expression in two B-complex disparate genetically inbred Fayoumi chicken lines following Eimeria maxima infection. Poultry Science 87, 433–443.
Lee, K., Lillehoj, H.S., Li, G., Park, M.S., Jang, S.I., Jeong, W., et al., 2011a. Identification and cloning of two immunogenic Clostridium perfringens proteins, elongation factor Tu (EF-Tu) and pyruvate:ferredoxin oxidoreductase (PFO) of C. perfringens. Research in Veterinary Science 91, e80–e86.
Lee, K.W., Lillehoj, H.S., Jeong, W., Jeoung, H.Y., An, D.J., 2011b. Avian necrotic enteritis: experimental models, host immunity, pathogenesis, risk factors, and vaccine development. Poultry Science 90, 1381–1390.
Lee, K.W., Lillehoj, H.S., Park, M.S., Jang, S.I., Ritter, G.D., Hong, Y.H., et al., 2012. Clostridium perfringens alpha-toxin and NetB toxin antibodies and their possible role in protection against necrotic enteritis and gangrenous dermatitis in broiler chickens. Avian Diseases 56, 230–233.
Lee, S.H., Lillehoj, H.S., Jang, S.I., Lee, K.W., Bravo, D., Lillehoj, E.P., 2011c. Effects of dietary supplementation with phytonutrients on vaccine-stimulated immunity against infection with Eimeria tenella. Veterinary Parasitology 181, 97–105.
Lee, S.H., Lillehoj, H.S., Jang, S.I., Lillehoj, E.P., Min, W., Bravo, D.M., 2013. Dietary supplementation of young broiler chickens with Capsicum and turmeric oleoresins increases resistance to necrotic enteritis. British Journal of Nutrition 110, 840–847.
Lee, S.H., Lillehoj, H.S., Jang, S.I., Jeong, M.S., Xu, S.Z., Kim, J.B., et al., 2014. Effects of in ovo injection with selenium on immune and antioxidant responses during experimental necrotic enteritis in broiler chickens. Poultry Science 93, 1113–1121.
Leeson, S., Namkung, H., Caston, L., Durosoy, S., Schlegel, P., 2008. Comparison of selenium levels and sources and dietary fat quality in diets for broiler breeders and layer hens. Poultry Science 87, 2605–2612.
Lillehoj, H.S., Lee, K.W., 2012. Immune modulation of innate immunity as alternatives-to-antibiotics strategies to mitigate the use of drugs in poultry production. Poultry Science 91, 1286–1291.
Madison, M.N., Kleshchenko, Y.Y., Nde, P.N., Simmons, K.J., Lima, M.F., Villalta, F., 2007. Human defensin alpha-1 causes Trypanosoma cruzi membrane pore formation and induces DNA fragmentation, which leads to trypanosome destruction. Infection and Immunity 75, 4780–4791.
Marsh, J.A., Dietert, R.R., Combs, G.F., Jr., 1981. Influence of dietary selenium and vitamin E on the humoral immune response of the chick. Proceedings of the Society for Experimental Biology and Medicine 166, 228–236.
Ministry of Agriculture, 2010. Ministry of Agriculture of People's Republic of China Bulletin No. 1224–2010. The safety use standard of feed additives. Beijing, China.
Mot, D., Timbermont, L., Delezie, E., Haesebrouck, F., Ducatelle, R., Van Immerseel, F., 2013. Day-of-hatch vaccination is not protective against necrotic enteritis in broiler chickens. Avian Pathology 42, 179–184.
Muller, P.Y., Janovjak, H., Miserez, A.R., Dobbie, Z., 2002. Processing of gene expression data generated by quantitative real-time RT-PCR. Biotechniques 32, 1372–1374, 1376, 1378–1379.
Park, S.S., Lillehoj, H.S., Allen, P.C., Park, D.W., FitzCoy, S., Bautista, D.A., et al., 2008. Immunopathology and cytokine responses in broiler chickens coinfected with Eimeria maxima and Clostridium perfringens with the use of an animal model of necrotic enteritis. Avian Diseases 52, 14–22.
Pavlata, L., Pechová, L.M.A., Dvořák, R., 2012. Comparison of organic and inorganic forms of selenium in the mother and kid relationship in goats. Czech Journal of Animal Science 57, 361–369.
Peng, X., Cui, H., Yuan, J., Cui, W., Fang, J., Zuo, Z., et al., 2011a. Low-selenium diet induces cell cycle arrest of thymocytes and alters serum IL-2 content in chickens. Biological Trace Element Research 144, 688–694.
Peng, X., Cui, H.M., Deng, J., Zuo, Z., Cui, W., 2011b. Low dietary selenium induce increased apoptotic thymic cells and alter peripheral blood T cell subsets in chicken. Biological Trace Element Research 142, 167–173.
Peng, X., Cui, H., Fang, J., Zuo, Z., Deng, J., Pan, K., et al., 2012. Low selenium diet alters cell cycle phase, apoptotic population and modifies oxidative stress markers of spleens in broilers. Biological Trace Element Research 148, 182–186.
Prescott, J.F., 1979. The prevention of experimentally induced necrotic enteritis in chickens by avoparcin. Avian Diseases 23, 1072–1074.
Ramasamy, K.T., Verma, P., Reddy, M.R., 2012. Differential gene expression of antimicrobial peptides beta defensins in the gastrointestinal tract of Salmonella serovar Pullorum infected broiler chickens. Veterinary Research Communications 36, 57–62.
Sumners, L.H., Cox, C.M., Kim, S., Salevsky, J.E., Siegel, P.B., Dalloul, R.A., 2012. Immunological responses to Clostridium perfringens alpha-toxin in two genetically divergent lines of chickens as influenced by major histocompatibility complex genotype. Poultry Science 91, 592–603.
Tanaka, T., Rahman, M.M., Battur, B., Boldbaatar, D., Liao, M., Umemiya-Shirafuji, R., et al., 2010. Parasiticidal activity of human alpha-defensin-5 against Toxoplasma gondii. In Vitro Cellular & Developmental Biology Animal 46, 560–565.
Timbermont, L., Haesebrouck, F., Ducatelle, R., Van Immerseel, F., 2011. Necrotic enteritis in broilers: an updated review on the pathogenesis. Avian Pathology 40, 341–347.
Yoon, I., Werner, T.M., Butler, J.M., 2007. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. Poultry Science 86, 727–730.
Yuan, D., Zhan, X.A., Wang, Y.X., 2012. Effect of selenium sources on the expression of cellular glutathione peroxidase and cytoplasmic thioredoxin reductase in the liver and kidney of broiler breeders and their offspring. Poultry Science 91, 936–942.